SUB- AND SUPERCRITICAL FLUID PROCESSING OF AGRIMATERIALS: EXTRACTION, FRACTIONATION AND REACTION MODES

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1. Introduction

The processing of agrimaterials utilizing sub- and supercritical fluids is one of the more challenging and time-honored applications of the technology. Agrimaterials can by definition include food, natural products, and nutraceuticals; however it is probably raw agricultural materials that encompass and present so many undefined variables to the technologist attempting to use critical fluid processing. Agrimaterials are "nature" in its most raw form, and the application of critical fluids for unit operations must be able to respond to a wide variation in substrate moisture content , molecular composition, and physical or seasonal morphology. In these cases, a unit operation like supercritical fluid extraction (SFE) will not be conducted under idealized conditions, i.e, those that are used to measure solute solubility in a laboratory environment. Such complications however have not limited the application of critical fluid technology to agrimaterials, since some of the most often cited and commercially-successful uses of the technology occur in this area.

In this chapter, we shall explore why several unit operations using critical fluids, separately or coupled, are frequently needed to produce a desired end product. Figure 1 depicts several process sequence possibilities that could use supercritical fluids for isolating or synthesizing the desired end products. SFE can be used to directly produce extracts or products. For example, the SFE of coffee beans results in a product that has been decaffeinated [1] for consumer use, while alternatively SFE can produce an extract from hops [2] that has commercial utility. However, such ideal scenarios are the exception to the rule, and more recently other applications of supercritical fluids have been explored which utilize supercritical fluid fractionation (SFF) or supercritical fluid reactions (SFR) to affect the desired end result.

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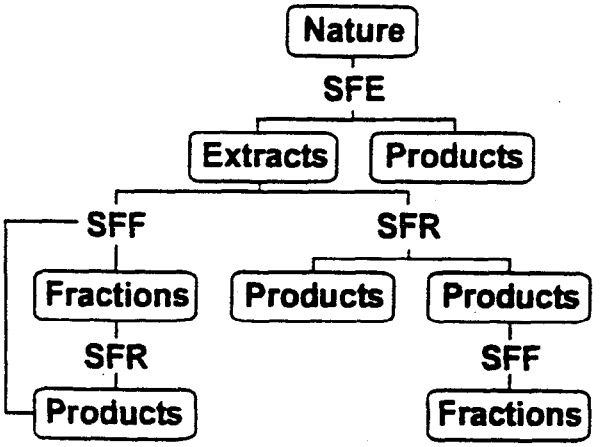

Figure 1. Processing sequences utilizing critical fluid media for isolating or synthesizing end products.

SFF or SFR an be applied directly to a natural substrate [3], but it is more likely as suggested in Figure 1, that they will be applied as unit operations after some preliminary extraction step, either critical fluid or conventional liquid, to produce a more definitive fraction or product. It is this increasing vista for critical fluid technology that we will explore in this chapter, using examples from the author's research and the literature, to illustrate the extensive application of critical fluids to the entire processing scheme for natural and agricultural products.

2. Optimizing SFE for Processing Agrimaterials

2.1. FLUID PROCESSING PARAMETERS

Optimization of SFE for the processing of natural materials requires adjustment of the extraction pressure, temperature, and fluid flow rate to achieve the desired end result. The author has advocated the knowledge or experimental determination of four phenomena which are essential for conducting a successful SFE. These are the:

Solute Miscibility Region
Fractionation Range for the Solutes
Solute Solubility Maxima
Solute Physical Properties

The solute miscibility region is the pressure and temperature at which the solute(s) initially become dissolved in the critical fluid, hence this bears a close approximation to the critical loci mentioned earlier in previous chapters. However, critical loci are composition dependent [4] and subject to the perturbation of coextractives and other variables, such as moisture, which occur in natural matrices. Determination of the solute miscibility region is also dependent on the method of measurement and its inherent sensitivity [5]. For example, the onset of solute miscibility into the critical fluid is often assessed gravimetrically, in keeping with the need to isolate actual material from the SFE. In practice, dissolved solutes that exhibit significant differences in miscibility pressures or temperatures maybe separable by adjustment of these variables, although it is rare to find a case where some degree of cross contamination does not occur (coextraction).

The fractionation range is defined by operating over a selected range of pressures and temperatures (i.e., densities) that permit a differentiation to be made between the solubility of the solutes in the critical fluid media, or some similar physical or chemical property (i.e., vapor pressure), so that some fractionation of the dissolved mixture of components can be affected. In terms of thermodynamic notation, this often corresponds to the "crossover region" [6] where an adjustment in operational pressure or temperature changes the relative selectivity of one solute over that of another. This produces enrichments of one or more components over that of another component(s), but rarely results in a high degree of solute purification unless the mixture has a simple molecular composition (which is rarely the case in natural or agricultural products). Such resultant enrichment maybe adequate for producing commercially-useful products or tailoring the physical properties of the final or fractionational extract [7].

The solubility maxima of solutes in a critical fluid are obviously important since they often define the conditions consistent with the highest throughput during SFE. Often determined for a pure solute in a pure critical fluid [8], they allow the practicing technologist to choose conditions where the maximum solubilization of the solute can occur. Such maxima have been recorded for both organic and inorganic solutes in a variety of fluid systems [9]. Giddings et al. [10] have approximated this condition using the "solubility parameter theory" showing that maximum solubility of a solute occurs when the solubility parameter of the solute and the critical fluid are nearly identical. However, caution should be exercised, since recovery of a solute from a natural material is highly dependent on the flux of the solute removed under specific conditions, and the flux is a complex function of both solubility and diffusion (i.e., mass transfer) in the critical fluid medium.

The physical properties of the solutes also play a crucial role in critical fluid extraction processes, particularly with regard to their molecular structure and temperature-dependent properties. The author has offered some insight into predicting solute solubility in supercritical fluids based upon a correlation between a molecular group structure contribution-solubility parameter correlation [11]. Suffice to say, the introduction of polar functional groups into a compound usually results in the need for a higher extraction pressure and/or temperature [12]. The vapor pressures of the solutes to be extracted or

separated also play a key role in the partitioning of the solutes into the critical fluid phase. This becomes apparent in the previously-mentioned "crossover" region where a solute's solubility in the critical fluid is no longer dependent on the density of the critical fluid as the extraction temperature is increased, i.e., the solute's solubility increases as the density of the extraction fluid is decreased. This effect can become quite significant in the SFE of oils from natural products [13]. The effect of differential vapor pressure is often used in SFF columnar processes to affect the separation of different solutes from each other in the critical fluid.

2.2 THE ROLE OF SOLUTE SOLUBILITY IN SFE

The influence of a solute's solubility in a critical fluid on optimizing SFE is apparent in setting the conditions for extraction and separation of the solute from the critical fluid, the time and quantity of fluid required for conducting the SFE, as well as the occurrence of coextractives (both wanted and unwanted) in the final extract. Two contrasting cases are cited in Figures 2 and 3, respectively, the solubility of triglycerides (the major constituents of fats and oils) in supercritical carbon dioxide (SC-CO_2), and the solubility of water in SC-CO_2. The solubility map shown in Figure 2 shows the influence of pressure for specific isotherms on the weight percent of triglycerides in SC-CO_2. One can see that by raising both the temperature and pressure, significant quantities of triglyceride can be solubilized in SC-CO_2. The observed trends in triglyceride solubility in SC-CO_2 can be used as the basis not only for extracting the target solute, but also to affect separation from the critical fluid phase. For example, in Figure 2, if one chooses to operate along the 80°C isotherm and decrease the pressure from approximately 770 bar to 700 bar, a 12 weight percent drop in solubility can be made to occur under these conditions (CT). Likewise, by electing to operate at constant pressure (CP), one can also raise or lower solute solubility in the SC-CO_2 by changing the temperature from 60°C to 80°C, or vice versa, and achieve a 17 weight percent differential in solubility. Hence, obviously there are advantages to adjusting both pressure and temperature to extract and precipitate a solute in a cyclic SFE process.

The solubility trends for another ubiquitous substance in natural products, water, is illustrated in Figure 3 [14]. Here the mole percent of water dissolved in SC-CO_2 as a function of pressure and temperature is shown. The solubility trends as a function of pressure are relatively monotonic and the amount of dissolved water in SC-CO_2 is a slight function of temperature. Contrasting the weight percent solubility of water versus that exhibited by triglycerides in SC-CO_2, one certainly sees the effect of solute polarity on the resultant solubility. This slight solubility of water in SC-CO_2 can have some profound effects in SFE and SFR as will be shown later.

2.2.1. Practical Implications of Solubility in SFE.

One of the more profound effects of solute solubility trends in supercritical fluids is exhibited by the "course" of an extraction as a function of time or volume of fluid passed

through the extractor. This is illustrated in Figure 4 for the extraction of oil from the evening primrose seed [15] as a function of pressure, mass of CO_2 utilized, and extraction time. The mass of oil recovered in grams increases with both mass of CO_2 used and

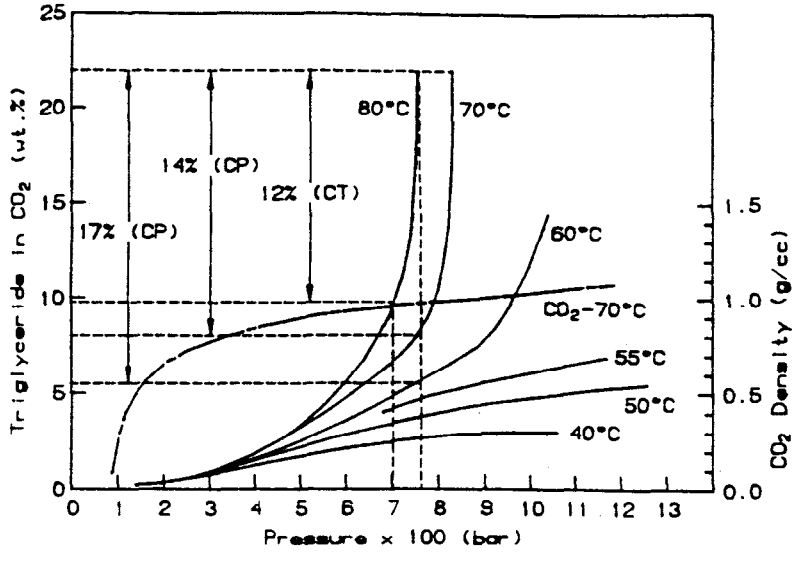

Figure 2. Solubility of triglycerides in SC-CO_2 as a function of temperature and pressure.

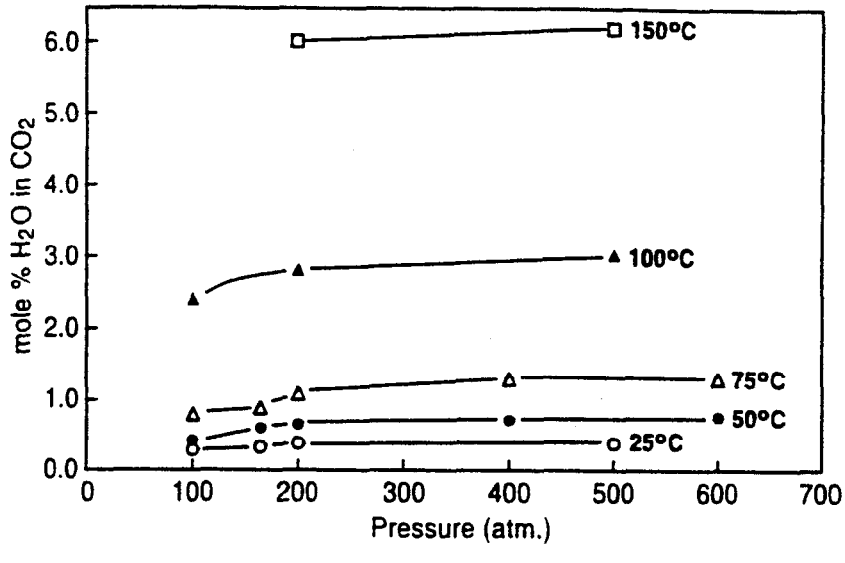

Figure 3. Solubility of water in SC-CO_2 as a function of temperature and pressure.

extraction time, but the rate of oil removal from the crushed seed matrix varies depending on the extraction pressure that is chosen. At 20MPa, the oil removed is a linear function of time or mass of CO_2; however by increasing the extraction pressure, one increases the solubilization of the oil in the SC-CO_2 and the extraction proceeds much more rapidly, requiring less time and quantity of CO_2. The initial slopes of the extraction curves are a quasi-equilibrium measure of solute solubility in the supercritical fluid (weight percent in the case illustrated), and the steepness of the initial slope is a measure of the relative dissolving power of the CO_2 for the oil (triglycerides) in the evening primrose seed.

This linear increase in amount of solute extracted as a function of mass of the extracting fluid gives way to a transitional region where the rate of extraction curve starts to become influenced by kinetic factors which inhibit the removal of the oil from the natural product matrix. Finally, the SFE enters an entirely mass transfer-controlled region characterized by an asymptotic behavior. In this region, the technologist is faced with the choice of how "complete" an extraction they wish to achieve. For example, if 99% removal of the oil will suffice, then the SFE can be truncated earlier than if 99.9% of the oil is desired for removal. Rate of extraction curves, such as illustrated in Figure 4, can be very diagnostic since they define some of the practical aspects of conducting a SFE. Certainly for the example cited in Figure 4, there is not much advantage operating above 50 MPa and for more than 40 minutes extraction time. Modeling of these extraction rate curves has been studied by numerous investigators [17-19] and excellent results have been achieved.

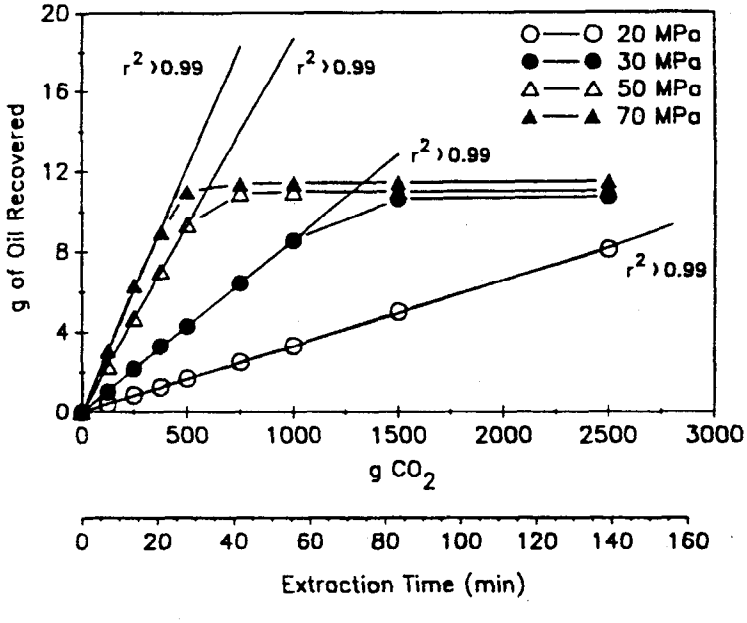

Figure 4. Extraction of evening primrose oil with SC-CO_2 at 40°C and different pressures.

However, a note of caution should be exerted here; curves such as illustrated in Figure 4 do not necessarily always indicate that all of the extractable product has been removed from the sample matrix. Favati [20] has shown that curves such as those in Figure 4 can be recorded, where upon by comminuting the "extracted" matrix, further extract can be removed. This is usually observed as a vertical displacement of the curves noted in Figure 4 [21].

Another way of viewing the course of a SFE is by recording the "differential" solute solubility as a function of extraction time or mass of fluid passed through the extractor. Here the weight percent of solute collected per unit time is computed and plotted versus the mass of fluid utilized. This approach is illustrated in Figure 5 for cottonseed oil SFE under two different extraction conditions. The dashed vertical lines shown in Figure 5 are equivalent to the mass of fluid contained in one extractor volume. Clearly, Figure 5 shows that conducting SFE at 12,000 psi and 80°C results in quicker completion of the extraction versus the result obtained at 8000 psi and 50°C. In the former case, only three extractor volumes of fluid are required to complete the extraction, while in the lower temperature and pressure case, over seven extractor volumes are required.

It is interesting to note that the results illustrated in Figure 5 bear a close resemblance to solute chromatographic profiles as they elute from a chromatography column. The higher pressure and temperature case resembles an elution chromatographic profile where the solute is more dilute in the carrier "fluid". However at the lower extraction pressure and temperature, the differential profile is similar to a frontal elution plateau, where the solute has reached saturation in the carrier fluid due to solubility limitations. Interestingly, chromatographic modeling of SFE differential and integral profiles has been described in a often-ignored paper by Goodrum et al. [22].

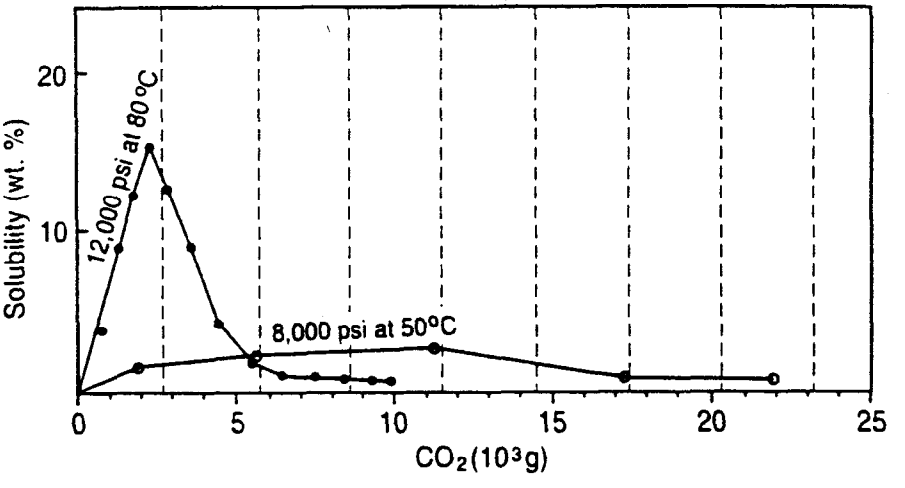

Figure 5. Weight percent solubility of cottonseed oil as a function of mass of SC-CO_2 passed through the seed bed.

2.2.2. *The Effect of Flow Rate on SFE.*

The effect of fluid flow through the extraction vessel can have a significant effect on the time of the SFE, particularly if the extraction conditions have been optimized with respect to solute solubility, etc. This is illustrated in Figure 6 for evening primrose oil extraction as a function of the reduced variable, mass of extract/mass of seeds (m_{ex}/m_{seeds}) versus extraction time. Although the presented data are for the linear, solubility-controlled lower pressure and temperature region (20MPa, 40°C), they clearly show that tripling the mass flow rate results in a corresponding decrease in extraction time for the collection of an equivalent amount of extract. By using a different reduced variable on the horizontal axis of Figure 6, namely the mass of fluid (CO_2) divided by the mass of seeds in the extractor bed, (m_{CO2}/m_{seeds}) [23], the different curves associated with different flow rates merge, illustrating that one of the most critical variables in attaining a fast SFE is the mass of fluid passed through the extraction vessel.

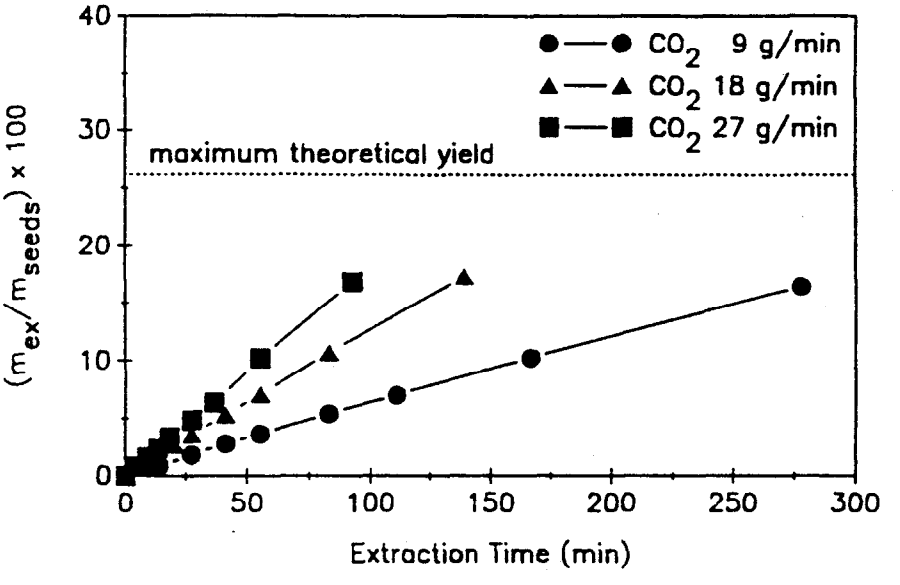

Figure 6. Influence of CO_2 mass flow rate on the rate of extraction of evening primrose oil at 20 MPa and 40°C.

To illustrate the practical application of fluid flow rate, the required efficiency of extraction, and other operational SFE parameters, data are given in Table 1 for the pilot plant extraction of soybean flakes. Extraction time, CO_2 flow rate, weight percent of oil recovered based on the initial weight of the flake charge into the extractor, and several weight percent data for residual oil in the extracted flakes are presented. It is obvious from

the data in Table 1 that increasing the flow rate can lead to a very rapid extraction (ten minutes) and high recovery of oil from the flake matrix. Results at intermediate flow rates and extraction times illustrate that there is some trade-off between attaining the highest possible efficiency of extraction and material throughput using SFE.

It is interesting to note that these "batch" extractions were each performed on separate batches of soybean flakes using a recycle SFE system in our laboratory [24]. Note that for these extractions, oil removal was affected at 12,000 psi and 80°C, while phase separation of the oil from the compressed CO_2 was achieved at 2500 psi and 80°C based on data given in Figure 2. The reason for not affecting complete decompression of the oil-laden fluid phase is economical, since to totally decompress to render the CO_2 oil-free would be prohibitive energy-wise.

It should be noted however that on some natural substrates, too rapid a flow rate can lead to compaction of the bed with resultant channeling of the fluid, yielding an incomplete extraction. This is particularly true for substrates that have a high oil content (e.g., peanuts), which have a propensity to compact in the extractor. In conclusion, it is the author's opinion that the lack of adequate flow and pressure are probably the two most important factors that mitigate against attaining rapid and complete SFE.

TABLE 1. Examples of rapid seed oil extraction using SC-CO_2

Extraction Time Time (min)	CO_2 Flow Rate (lbs/min)	Weight % Oil Recovered	Residual Oil (wt.%)
25	0.66	20.1	-------
15	0.66	19.4	0.8
14	0.72	18.3	1.4
11	0.92	19.4	0.7
10	1.00	18.6	-------

3. Supercritical Fluid Fractionation (SFF)

SFE can be somewhat limiting with respect to its ability to fractionate solubilized m ixtures

of components. Density-based fractionations can be accomplished most successfully when there are large differences in the molecular weight or chemical properties of the solutes. Even then, coextraction of components is the rule rather than the exception.

Some examples of successful commercial selective extractions are the removal of caffeine from coffee or the solubilization of nicotine from tobacco; both accomplished on moist matrices to aid in selectively solubilizing the alkaloid component. Selective extraction has been demonstrated for the segregation of essential oil from other lipid components in natural extracts derived from fruits and for the separation of aroma components in cocoa butter from the base oil. Other "enrichment" SFE schemes that have been reported include the fractionation of carotenoid from leaf protein concentrate [25], the fortification of sterols in seed oils [26], and the isolation of lecithin (phospholipid-containing fraction) from triglycerides [27].

By employing an assisting mechanism in conjunction with SFE, higher resolution supercritical fluid fractionation (SFZ.) can be achieved. Examples of these mechanisms that have been used are packed fractionating columns [28] that are somewhat analogous to distillation columns. These units can be operated isothermally or employ temperature gradients [29]. Chromatographic fractionation has also been reported using both commodity and specialized sorbets [30] as packing. Recently, Sato et al. [31] have demonstrated that the principle of pressure-swing adsorption is compatible with the use of critical fluids. Stepwise let down of the pressure and/or temperature in a series of separator vessels is also a form of fractionation and has been widely applied to a number of agricultural products by Reverchon and associates [32]. Limited use has been made of the principle of specific molecular complexation to date [33], perhaps due to the need to provide a sufficient fluid density to overcome the strongly attractive forces that are characteristic of solute-complexing agent interactions. As a pedagogical device, we are going to examine several approaches for fractionation that we have employed in our laboratory, to illustrate the principles of SFF, and its attendant advantages and limitations.

3.1. EXAMPLES OF SFF PROCESSES

3.1.1. The Continuous Isolation of a Lecithin Fraction from Soybean Oil

The extraction of a seed oil with SC-CO_2 permits the solubilization of most lipid-based moieties contained in the seed with the exception of polar lipids such as phospholipids. In the oleochemistry industry, this fraction is frequently referred to as lecithin and it is sparingly soluble in SC-CO_2. Hence, lecithin, which has considerable commercial value, is left behind in the protein seed matrix after extraction with SC-CO_2 [34]. This phospholipid-laden fraction can be dissolved in SC-CO_2 by using ethanol as a cosolvent.

Isolation and enrichment of lecithin from the neat seed oil can also be affected by using SC-CO_2 in a continuous countercurrent fashion to dissolve the oil in the fluid phase, while the insoluble lecithin components drop to the bottom of the column. The design of such a unit is shown in Figure 7 in which refining vessel is a column packed with asegmented

distillation type of packing material. The soybean oil is fed into the refining vessel (kept at 8,000 psi and 70°C) by a high pressure liquid pump, and is counter currently contacted, with the SC-CO_2 coming up from the bottom of the vessel. Separation of the sparingly-soluble lecithin from the soybean oil triglycerides takes place in the refining vessel, resulting in what is referred to as a "degummed" oil as a top product. The insoluble lecithin fraction settles to the bottom of the refining vessel where it can be isolated after completion of the run by release of the pressure on the refining vessel. The resultant degummed oil is collected in the designated receiver vessel kept at about 2500 psi and 80°C (remember the conditions discussed in Section 2.2.2), and the partially depressurized CO_2 is sent back to the main compressor. The system in Figure 7 operates continuously; an advantage over a batch SFE process in terms of permitting the continuous feed of a preextracted oil and allowing the recovery of the lecithin fraction [35].

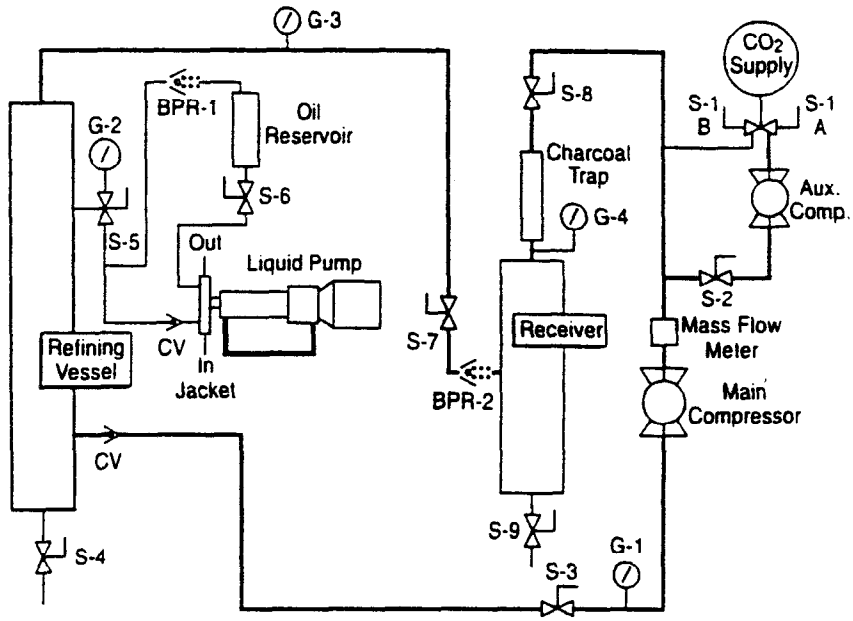

Figure 7. Continuous SC-CO_2 degumming system.

3.1.2. Thermal Gradient-Based Fractionation in a Packed Column

One of the earliest examples of a SFF process has made use of a thermal gradient to amplify the density differences in the critical fluid and the physicochemical properties of the solutes that are to be separated. Perhaps one of the initial uses of thermal gradients along with supercritical fluids is the "hot finger" approach used by Eisenbach [36] to fractionate fish oil ethyl esters into fractions having different physical properties and

chemical compositions. Today this has been taken to a high level of practice has typified by the studies of Nelson and coworkers [37], and others. For illustrative purposes, we should like to discuss our recent studies [38] on the fractionation of glyceride mixtures using a thermal gradient, packed fractionating column.

Figure 8 shows how the thermal gradient column works. Carbon dioxide is delivered to the base of the column by a gas booster pump into a preheated vessel that converts it to SC-CO_2. The SC-CO_2 is then subjected to a steadily increasing temperature through the application of thermal gradient in four separately heated zones (60, 70, 80, 90°C). This changes the density of the CO_2 its solubility characteristics, and mass transport properties; longitudinally along the column. The vapor pressures of the respective mixed glyceride classes we are trying to separate also play a key role, since a light over heavy selective results, based on this factor.

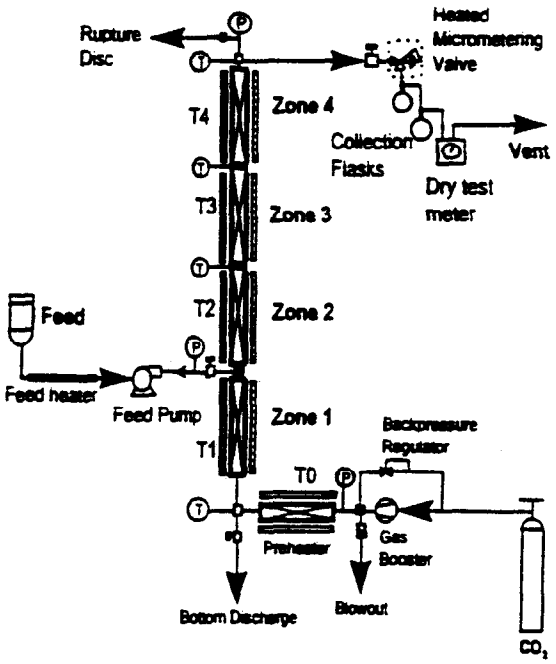

Figure 8. Schematic of thermal gradient packed column fractionation system.

Typically, a mixture of glycerides containing approximately 35-45 weight percent monoglyceride content is fed into the column right above the first heated zone. The column is packed with Propack, a commercially-available distillation wire mesh packing.

It should be noted with interest, that the above-quoted monoglyceride-containing mixture is available commercially, but it can also be produced by high pressure synthesis in the presence of SC-CO_2 , or by enzymatic-initiated catalysis in the presence of SC-CO_2 [39]. The batch or continuously injected glyceride mixture then contacts the upward rising SC-CO_2 and proceeds to be fractionated along the length of column; the monoglyceride components be continually enriched at the top of the column (due largely to vapor pressure differences). This enriched fraction can be collected continuously or on a batch basis at the top of the column by depressurization of the critical fluid.

The variation is some key properties of SC-CO_2 as a function of temperature along the column are illustrated in Figure 9. Here we see a drop in the CO_2 density as temperature is increased regardless of the column pressuret. Correspondingly, the solubility parameter of the CO_2 decreases with temperature [40], as does the viscosity. Solute vapor pressures also increase at the higher temperatures and this is most noticeable for the lower molecular weight components (i.e., monoglycerides) relative to the di- and triglyceride moieties.

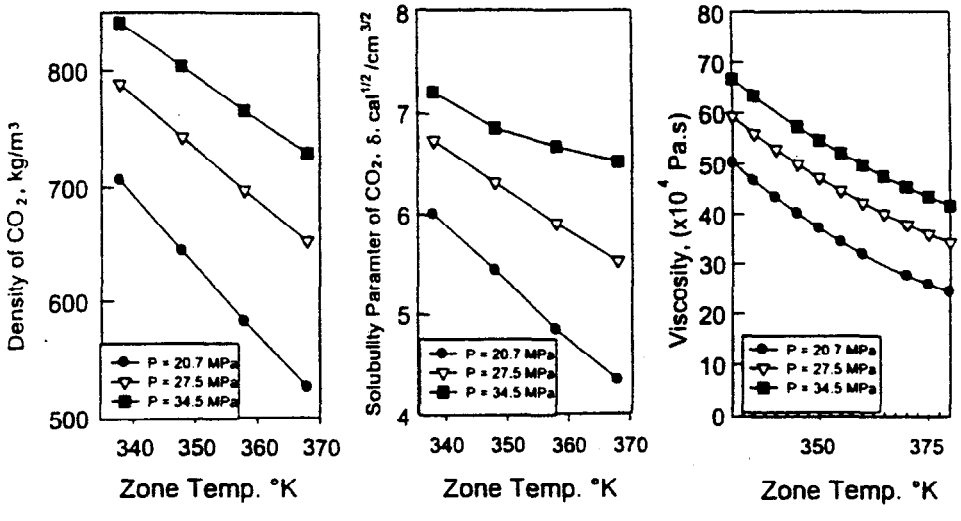

Figure 9. Changes in properties of SC-CO_2 along the fractionating column.

Figure 10 shows the effect of column operating pressure on the composition of the resultant top.product. One can see that by operating at a pressure slightly in excess of 2500 psi, products having a monoglyceride content of over 90 weight percent can be achieved. This valuable top product is equivalent to a similar product derived by vacumn distillation and is of high commercial value. Figure 10 also illustrates that by selecting a higher operating pressure, it is possible to fractionate a "designer" product having a specific

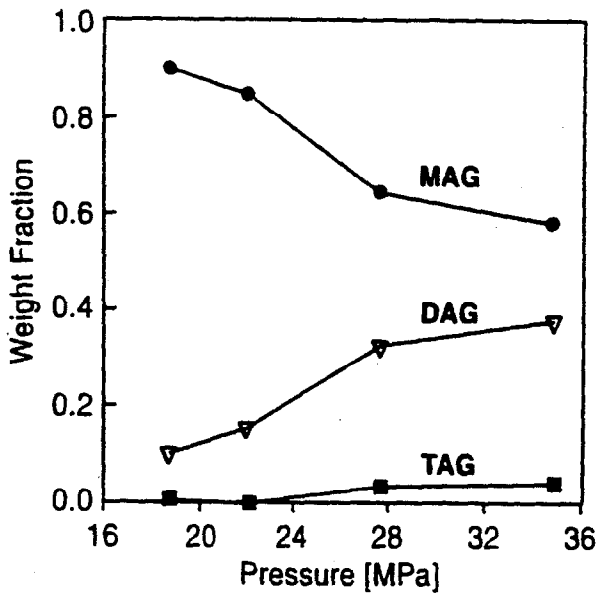

Figure 10. Effect of pressure on the columnar fractionation of glyceride mixtures.

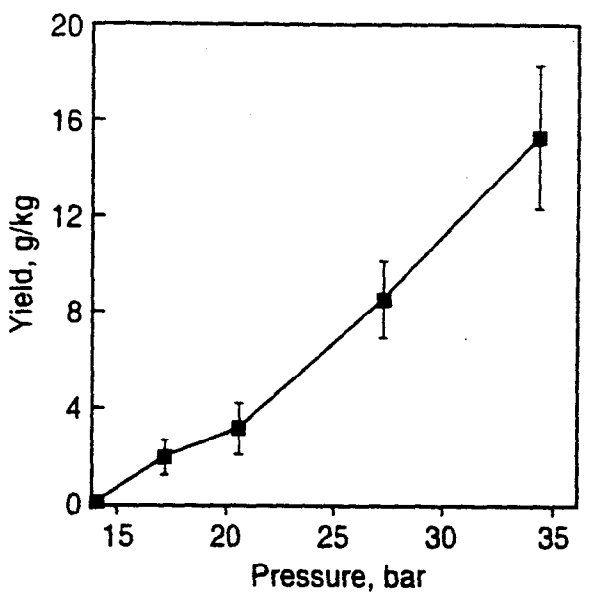

Figure 11. Effect of pressure on the top product yield during thermal gradient column fractionation of glyceride mixtures.

This valuable top product is equivalent to a similar product derived by vacumn distillation and is of high commercial value. Figure 10 also illustrates that by selecting a higher operating pressure, it is possible to fractionate a "designer" product having a specific glyceride composition. When the results shown in Figure 10 are combined with the gravimetric yield data as a function of pressure shown in Figure 11, one sees that there is a tradeoff between resolution or fractionation of the feed mixture and throughput. This verifies an old maxim of separation science [41] that resolution suffers as solute concentration increases. Therefore, the richest top product in monoglyceride content can only be achieved at the expense of total product throughput.

Finally, it is possible to achieve a high degree of separation between the individual glyceride classes (mono-, di-, triglycerides) using this column. Figure 12 shows the dynamic glyceride composition profile achievable under conditions of internal reflux caused by the thermal gradient. The horizontal axis shows that as one subtracts the amount of glyceride in the column over the amount fed to the column (B/F), that a 90 weight percent monoglyceride content product can be produced from the initial 50% of the mixture processed. Then the diglyceride components are fractionated off at a similar level of purity followed by the triglyceride components. This illustrates the power afforded by this particular approach for conducting SFF.

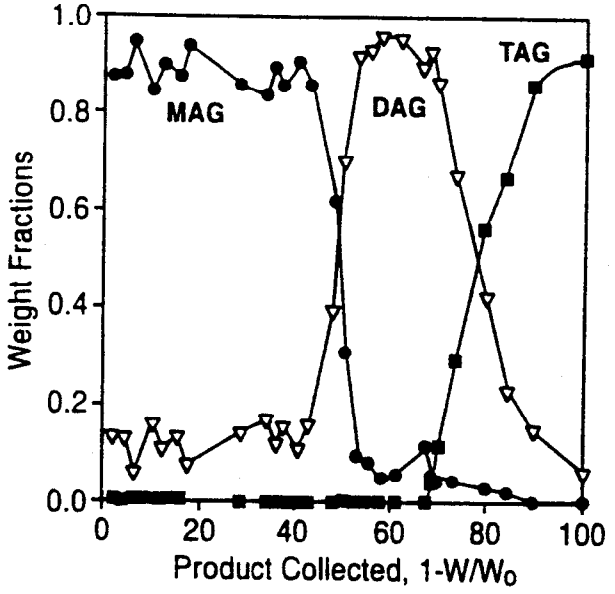

Figure 12. Dynamic glyceride composition profile in thermal gradient column during fractionation of glyceride mixtures.

3.1.3. SF. by Coupling SFE with Preparative SFC

Another approach to utilizing SF. is to couple it with a preliminary extraction step using critical fluid media. Here the separation specialist can amplify the impact of choosing the optimal SFE conditions with an additional SF. step to separate and enrich the desired components even further then could be accomplished using either step alone. An example from the author's laboratory [42] is illustrated in Figure 13 where natural antioxidants, tocopherols, found in low concentrations in many seed and natural product matrices, have been processed using these coupled critical fluid options. These naturally occurring tocopherols are shown structurally in Figure 14. They differ only slightly in molecular weight and in the position of the methyl groups on the aromatic ring structure.

The scheme depicted in Figure 13 suggests that a selective SFE step can be applied to extract just tocopherols and some lipid coextractive, in this case soybean oil. This initial step, starts to concentrate these high value chemicals from the matrix, while leaving the predominately-oil laden flakes available for extraction, perhaps by the SC-CO_2-based process described in Section 2.2.1. Hence, by utilizing the SC-CO_2-based process to deoil the flakes, a proteinaceous residue is left behind that has potential as either an animal or human food. The initially-derived extract, enriched in tocopherol content, is then transported to the top of a chromatographic column, whereby using SC-CO_2 as a eluent in conjunction with a commodity sorbent (silica gel), one can further enrich the tocopherol content of chromatographed extract by separating it from the background oil (triglyceride) content.

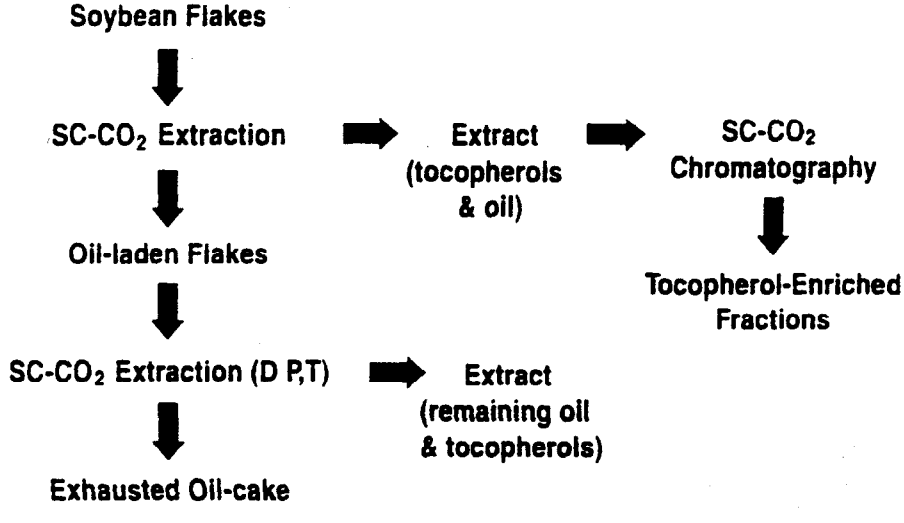

Figure 13. Tocopherol enrichment/fractionation by supercritical techniques.

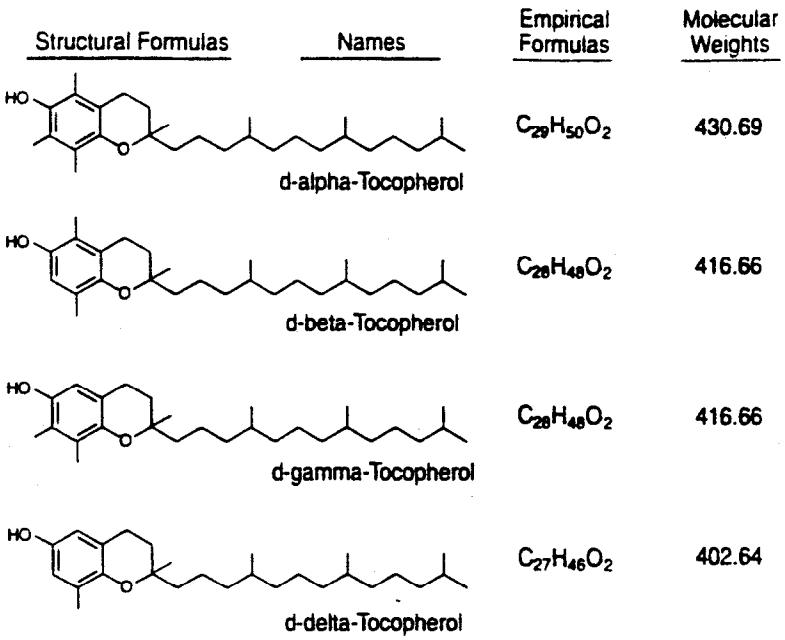

Figure 14. Molecular structure of tocopherols found in soybean oil.

This is accomplished using the apparatus shown in Figure 15. Here one can see the extractor vessel in which the soya flakes are placed ahead of the fluid delivery system components: the compressor and ballast tank. The initial extracts are collected as a function of time by passing the SC-CO_2 over the flake bed held in the extractor, and directly deposited on the chromatographic column. Then by switching the appropriate valves (V-2, V-3, etc.), the CO_2 flow can be diverted from passing through the extractor to passing through the chromatographic column, where the extracted tocopherols are further separated and concentrated in the receiver vessel (R). Pressure is maintained on the extractor bed and column by a pressure regulator (PR) and micrometering valve (MV), respectively.

The initial SFE stage is conducted using the parameters noted in Figure 16. This pressure (25 MPa) and temperature (80°C) were selected after several screening runs in which other conditions were also investigated. Figure 16 shows the percent recovery of the tocopherols and total oil as a function of total grams of CO_2 passed through the extractor bed over the total mass of the soybean flakes. Note that a worthwhile enrichment is occurring up to a reduced mass of approximately 65, where at this point the oil starts to coextract, significantly diluting the tocopherol-containing extract. At this point one would stop the SFE and divert the extract to the chromatographic column for further enrichment.

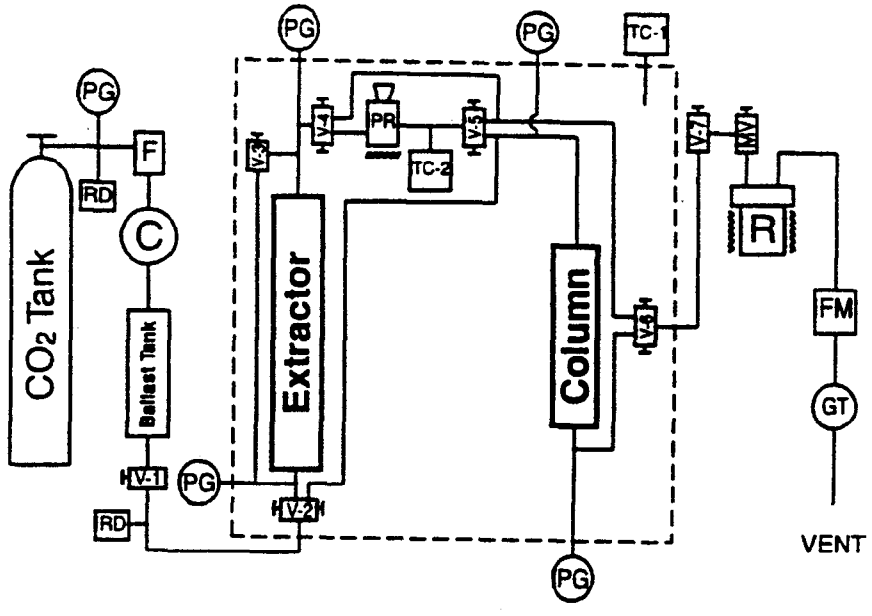

Figure 15. Schematic of SFE/SFC unit for enrichment of tocopherol fractionation from soya flakes.

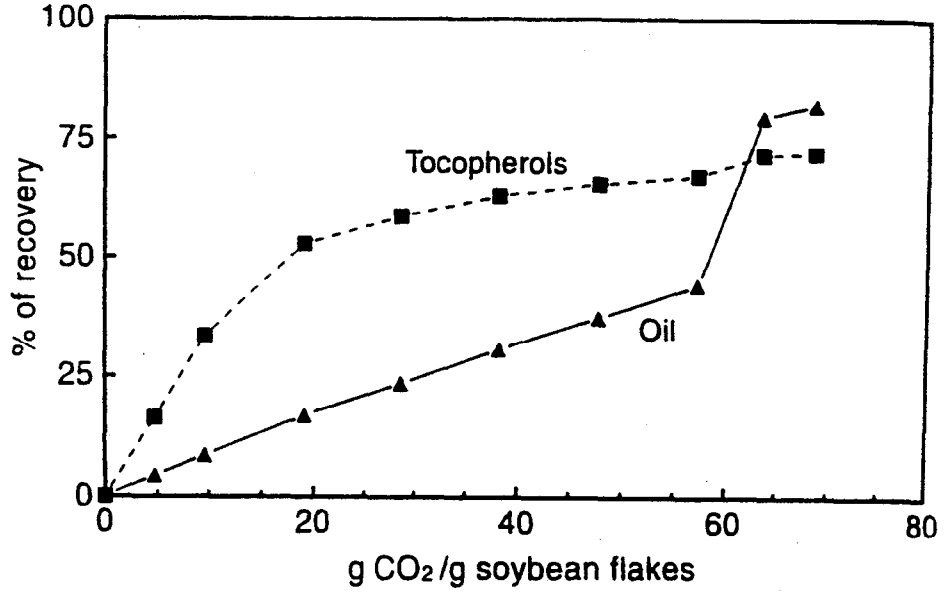

Figure 16. SC-CO_2 extraction of soybean flakes at 25MPa and 80°C.

The concentrating effect of this initial SFE stage is illustrated by Figure 17 where the analysis results show the higher levels of tocopherols in the sequentially collected extracts relative to their concentration in neat soybean oil. If one compares the top graph showing the enrichment of the tocopherols with the bottom graph which depicts the SFE of the oil, it is apparent that there is a point where further extraction is counter productive.

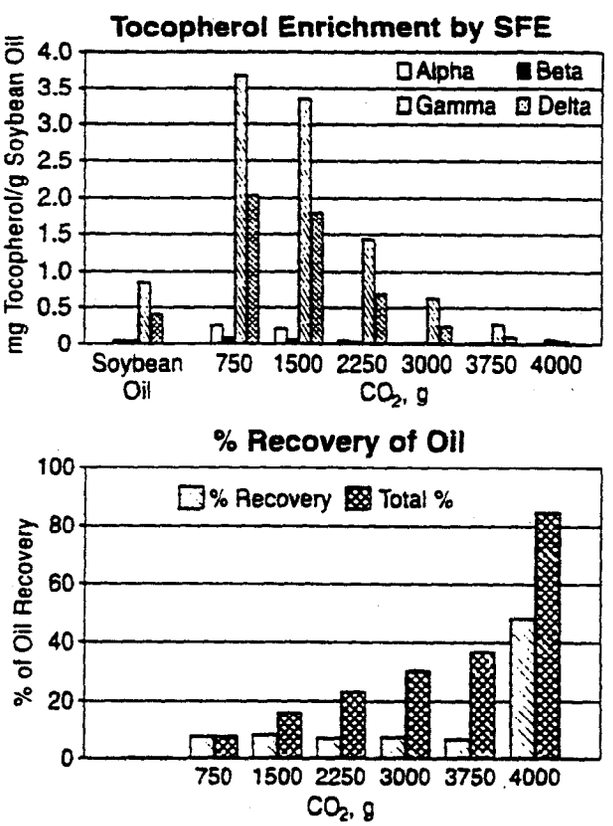

Figure 17. Tocopherol enrichment and wt. % recovery of oil from soya flakes as a function of mass of CO_2 passed through extractor.

The SFC stage, using silica gel as the sorbent, performed at 25 MPa and 40°C, results in further concentration of the tocopherol moieties. If collection of fractions occurs commensurate with elution of the tocopherols from the column, enrichments of individual tocopherols is possible. Data to support this view are presented in Table 2 where the enrichment factors for the four major tocopherols found in seed oil matrices are shown relative to the tocopherol concentration in the oil imbibed in the native seed matrix. The SFE stage produces enrichment factors raging from 4.33 to 1.83. By taking these fractions /and further enriching them by preparative SFC, the authors were able to achieve enrichment factors (from 30.8-2.4), considerably higher then those obtained by using optimized SFE alone.

TABLE 2. Enrichment of tocopherols from soybean flakes

Tocopherol	SFE	SFE + SFC
alpha	4.33	12.1
beta	1.83	2.4
gamma	3.94	15.0
delta	3.75	30.8

Another example of the above concept using tandem methods has recently been demonstrated in the author's laboratory for separation and enrichment of phospholipids (PPLs) from soya flakes. Phospholipids which are minor, high value constituents found in soybeans and other seed matrices, have limited solubility in neat SC-CO_2, however their solubility can be improved substantially by using ethanol as a cosolvent with SC-CO_2 [43]. The major individual phospholipids found in soybeans are shown in Figure 18. These individual moieties have common structural features, but different substantially in the various R groups (Figure 18). The chemical nature of the various R groups confer different polarities and chemical properties for the individual phospholipids ranging from acidic to basic to amphoteric behavior. However, despite these differences, they can all be solubilized in SC-CO_2/ethanol fluid mixtures.

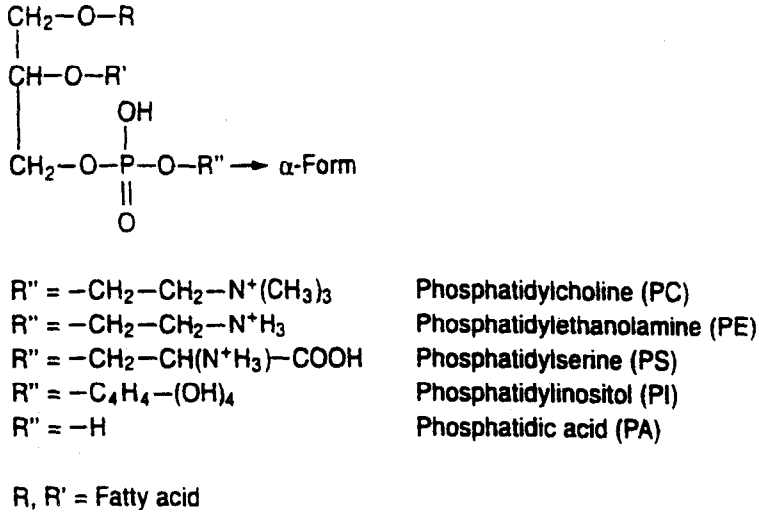

Figure 18. Molecular structure of phospholipids found in soya flakes.

The scheme for separating PPLs from the agrimaterial, soya flakes, is illustrated in Figure 19. Here the soybean oil can be exhaustively extracted using SC-CO_2, and the defatted flake matrix can then be subjected to extraction with SC-CO_2/ethanol to isolate the phospholipid-rich fraction. Initial experiments using this approach showed that selective enrichment of the PPLs was limited [27]; indeed the phospholipids could be extracted from the defatted flakes, but separation between the individual PPLs was lacking. Additional studies by Montanari et al. [44] showed that a refined SC-CO_2/ethanol extraction could serve to enrich phosphatidylcholine (PC) relative to the other three major phospholipids: phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI) and phosphatidic acid (PA). These enrichments are shown in Table 3 where the PPL composition of the resultant extract is tabulated versus the fluid density at 80°C for 10 mole percent ethanol in SC-CO_2. It appears that high concentrations of PC occur above 23.9 MPa and a further increase in fluid density diminishes the PC content of the extract slightly. It is also interesting to note, that the selectivity for PC suffers somewhat as larger amounts of PPLs are extracted (greater throughput), similar to trends noted using the thermal gradient fractionation column described in Section 3.2.2.

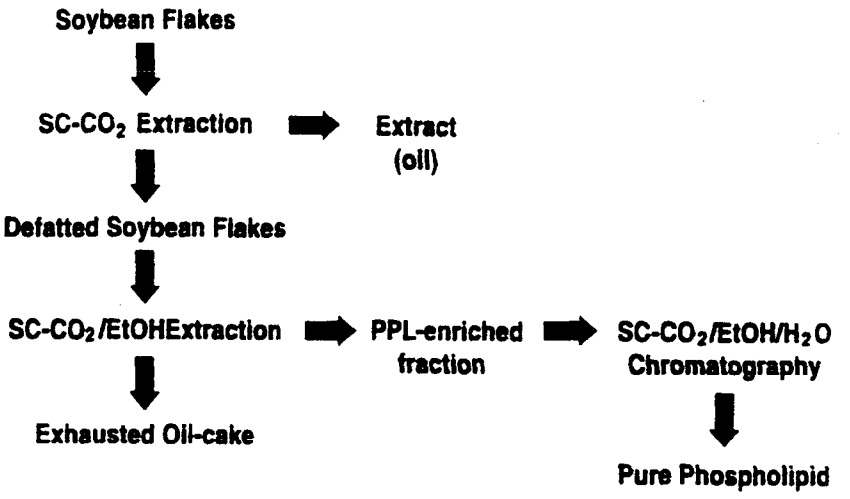

Figure 19. Phospholipid enrichment/fractionation by supercritical fluid techniques.

TABLE 3. Percent phospholipids in extracts from deoiled soybean flakes at 80°C as a function of pressure.

Phospholipid	16.6 MPa	23.9 MPa	40.7 MPa	68.9 MPa
Phosphatidylethanol-amine	44.1	18.5	20.8	25.1
Phosphatidylcholine	20.7	77.7	73.4	68.8
Phosphatidylinositol	35.2	2.8	1.8	3.6
Phosphatidic Acid	0.0	1.0	4.0	2.5

These SC-CO_2/cosolvent-based extractions of PPLs can also be improved upon by employing preparative SFC. This concept has been recently tested in the author's laboratory whereby a lecithin-based concentrate has been fractionated using silica gel (the same silica gel used for the tocopherol separations previously described). However, the presence of an adsorbent strongly adsorbs the PPL moieties, even when using SC-CO_2/ethanol mixtures as eluents. Elution of the PPLs from the silica gel can only be affected by using high pressures and larger amounts of ethanol as a cosolvent (perhaps more properly referred here as an eluent modifier). We have found that the triglyceride-based components in the PPL concentrate can be selectively eluted at 350 bar with neat CO_2, and even more rapid elution of these components can be achieved using the same pressure, but with a 10 volume percent of a 9:1/ethanol:water cosolvent. To achieve elution of the individual PPLs requires additional cosolvent (90:10 vol. %/ethanol:water) and 500 bars of pressure.

Some contrasting results obtained by high performance liquid chromatographic (HPLC) analysis of the obtained PPL fractions are shown in Figures 20a-c. Figure 20a shows the HPLC profile of the starting lecithin concentrate containing the four PPLs noted in Table 3. Figure 20b is the fifth fraction collected using the 25 vol. % modifier with SC-CO_2. In this case, both PI and PC have been enriched relative to the other PPL components in the lecithin concentrate. A latter collected fraction, Figure 20c, shows predominately only the appearance of PC, a valuable oleochemical that finds widespread use as a surfactant ingredient, in various food applications, and more recently in the formulation of liposomes. This once again illustrates the value of utilizing tandem, critical fluid-based processes to achieve high resolution when processing these complex agriculturally-derived materials.

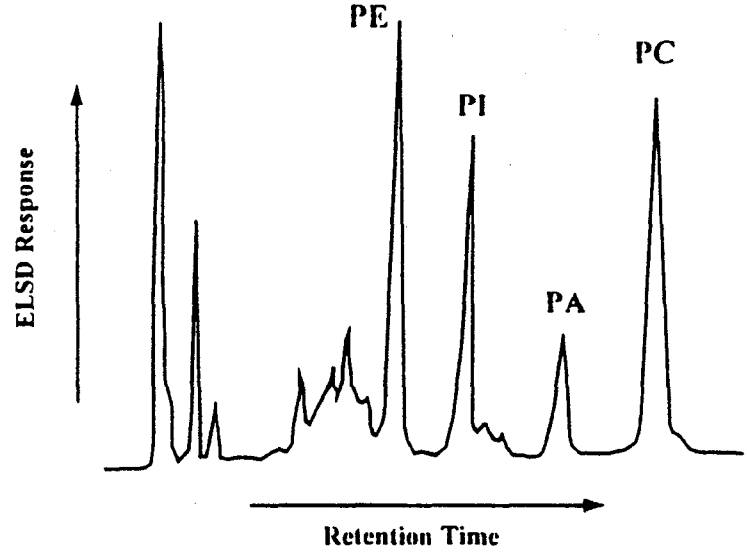

Figure 20a. HPLC profile of lecithin components prior to supercritical fluid processing.

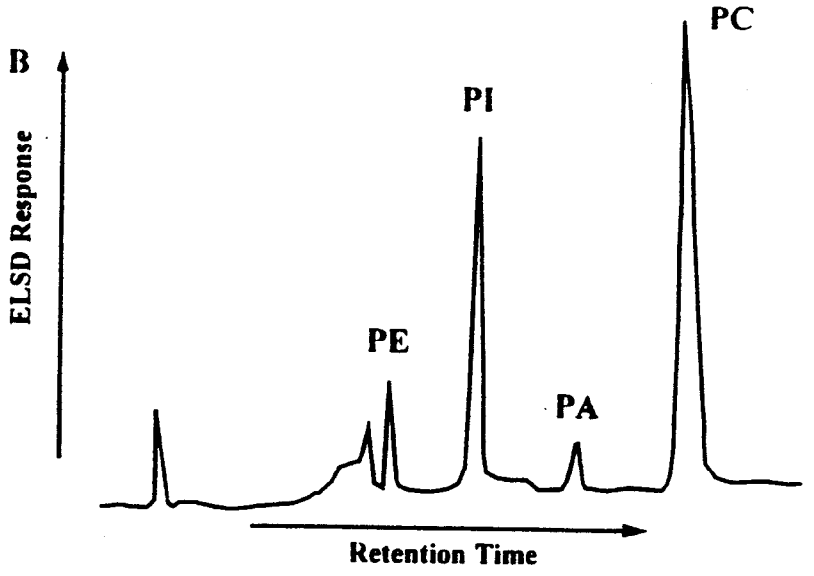

Figure 20b. HPLC profile of preparative SFC-extract (fraction #5) from lecithin.

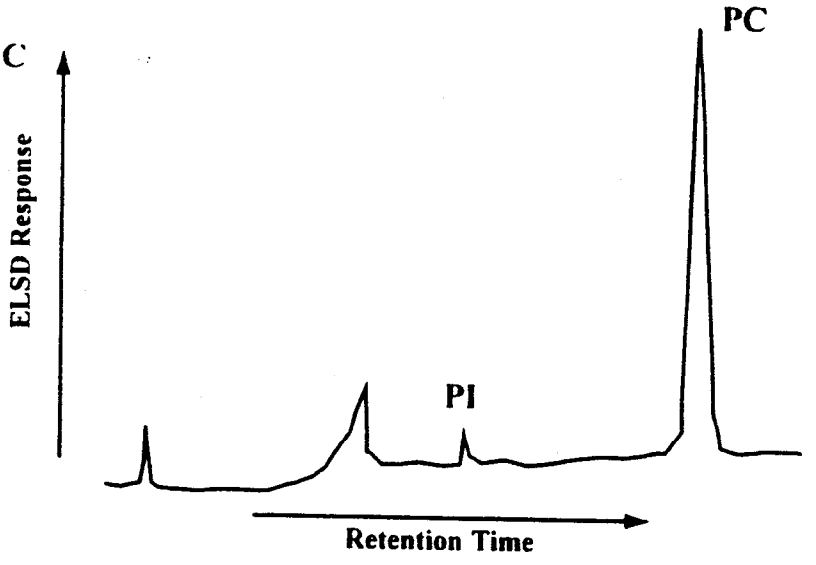

Figure 20c. HPLC profile of preparative SFC-extract (fraction #7) from lecithin.

4. Reaction Chemistry of Agrimaterials in Supercritical Media

Conducting reactions utilizing critical fluid media has been under extensive study over the past seven years [45]. Such conversions have been facilitated by using in particular SC-CO_2 as a catalytic agent [46], as a "solvent" medium for synthesis, and or in conjunction with condensed liquid media to improve the yield, selectivity, or kinetics of a reaction. Numerous types of reactions have been conducted with the aid of critical fluids, and a generic list is tabulated below:

Enzymatic
Heterogeneous Catalysis
Conversions in SC-H_2O
Polymerization
Pyrolytic
Photolytic
Reactions of Analytical Significance

The first three types of reactions: enzymatic, heterogeneous catalysis, and conversions in sub- and supercritical water; are of particular interest because of their application to agrimaterials.

Critical fluids offer some unique advantages when conducting reactions, including improvements in mass transfer of reactants and products, due to the improved diffusion coefficients exhibited by such solutes in the dense fluid media relative to the condensed liquid state. Since solute (reactant or product) solubilities are dependent on fluid density, one has potential control of the final product distribution by altering the temperature or pressure on the reaction system. This is even more so when reactions run are in liquid media, since synthesis in critical fluids allows control over the reaction rate by variation in the temperature, pressure, and flow of the critical fluid/ feed of reactants into the system. Additional attractive options include the possibility of performing conversions at low temperatures, in-situ regeneration of catalysts, and combining the reaction step sequentially with SFE or SF.. It should be noted that the Gibbs free energy of reaction is sensitive also to pressure as a variable as defined by Equation 1:

$$(\partial RT \ln K_x / \partial P)_T = \nabla V \qquad (1)$$

where K_x is the mole fraction equilibrium constant and ∇V is the excess partial molar volumes of the products over the reactants in the equilibrium mixture. Therefore, regardless of any benefits that adhere to conducting a reaction in critical fluid media, the application of pressure will have an influence on the reaction.

4.1. TRANSFORMATION OF LIPID-BASED AGRIMATERIALS VIA SFR

To provide some examples of utilizing supercritical fluid reactions (SFR) for the conversion of agrimaterials, we shall cite studies on the conversion of lipid-based materials of agricultural origin. Parameters which impact on converting lipid-based substrates in critical fluid media are tabulated below:

Pressure
Temperature
Phase Equilibria
Solute (Lipid) Solubility
Optimization of Reaction Conditions
Catalyst Type and Activity
Moisture Content of Substrate
Effect of Flow Rate
Throughput

The effect of some of these parameters is obvious, however several deserve additional comment. Phase equilibria and solute solubility relationships are important, not only with respect to assuring that adequate solute (reactant) solubility occurs in the critical fluid media, but that an adequate throughput of converted product is feasible to make the synthetic process viable and economical. Other important interrelationships are the optimization of reaction conditions via proper selection and activation of the catalyst (if required) and the moisture content of the substrate. Flow rate in tubular reactor systems is also critical, not only with respect to the critical fluid, but for the introduction of reactants and their solubilization into the critical fluid media. Flow rate is also linked to product throughput and must be optimized to allow proper kinetic conversion of the reactants.

One promising area for applying SFR for the conversion of lipid agrimaterials is the use of enzymes for accelerating such reactions as esterifications, transesterifications, oxidation, alcoholysis, and hydrolysis. All of these types of reactions have been shown to be feasible in SC-CO_2 and other critical fluids using both batch, stirred reactors and flow reactors [47]. Immobilized enzymes on porous supports for conducting conversions in a tubular flow reactor are particularly amenable for conversions using critical fluid media, since many of the variables can be altered and utilized for synthetic purposes. We have found Novozym SP 435 supported on a polyacrylic resin [48] to be particularly versatile for several synthetic options in SC-CO_2, including esterifications [49], transesterifications [50], alcoholysis [51], and interesterifications [52].

Jackson and King [50] have demonstrated the compatibility of transesterification on oils directly extracted from their seeds using the above enzyme in a flow reactor system. Trans- and simple esterifications can be conducted using Novozym SP 435 at pressures from 2500 - 5000 psi and temperatures from 40 - 70°C. The utilization of the higher

temperatures and pressures can reduce the service lifetime of the enzyme, but in most cases activity can be restored via hydration. An example of a SC-CO_2/Novozym SP 435 - based transesterification performed on olive oil in terms of the resultant fatty acid distribution profile is shown in Table 4. Here we see excellent agreement between the results achieved with synthesis in SC-CO_2 versus reported literature results. The formation of fatty acid methyl esters is so reproducible and quantitative, that it has served as a basis for analytical SFE/SFR methods developed in our laboratory [53].

Indeed, if the lipolysis is performed on corn and soybean oils, rather than olive oil, the fatty acid distributions when compared to those obtained via a classical derivatization method, methanolysis using BF_3, are excellent. These resultant fatty acid methyl ester (FAME) profiles can be used in nutritional analysis to evaluate the contribution of specific fatty acids to human metabolism. Lipolysis using the conditions that Jackson and King [50] have established can also be used successfully to methylate other lipid moieties, such as sterols and phospholipids. Such results pave the way for esterification of these compounds to other synthetic compounds having different fatty alcohol chain lengths. Recently, sterol esters have been synthesized by our research group using SC-CO_2 and various lipases. Partially dehydrated soapstock feeds containing fatty acids have also been esterified using the above condition; additional testimony to the general synthetic utility of Novozym 435 under supercritical fluid conditions, its general applicability to a variety of substrates, and tolerance of imbibed water in the SC-CO_2 fluid stream while synthesizing compounds from moisture-laden agricultural materials.

TABLE 4. Methyl ester composition from transesterification of olive oil in SC-CO_2 compared to literature values.

Fatty Acid	SC-CO_2	Literature
Palmitic	11.6	13.0
Palmitoleic	0.4	1.0
Stearic	4.9	2.6
Oleic	74.7	74.0
Linoleic	8.0	9.0
Linolenic	0.4	0.5

Conducting esterification reactions in supercritical fluid media offers a high degree of flexibility in "designing" a end product of a particular composition, and advantages over conducting the same reaction in liquid media. Table 5 shows some of the results for synthesizing the mono- and diester formed between lauric acid and 1,2 -propanediol using enzymatic-catalysis in SC-CO_2. For example, the yield of monoester formed between the reaction of lauric acid and 1,2 - propanediol is only 58% in n-hexane, while synthesis in SC-CO_2 at 2500 psi and 60°C yields the monoester in excess of 80%. Table 5 also shows that the selection of synthesis pressure affects the yield of mono- and diester as well as their respective ratios. These ratios of end products all tend to be higher via the supercritical fluid synthesis route than in n-hexane, although the diester content for the product mixture synthesized at 2500 psi and 50°C is identical to that found for the synthesis in n-hexane.

TABLE 5. Esterification between lauric acid and 1,2 - propanediol in SC-CO_2 and n-hexane.

Pressure (psi)	Monoester	Lauric Acid	Diester	Mono/Di Ratio
2500	80.9	8.4	10.7	7.5
4000	80.6	11.3	8.1	10.0
5800	74.2	18.7	7.0	10.6
n-hexane	58.2	31.0	10.8	5.4

Note that the optimum results for synthesizing either the mono- or di-ester do not occur at higher pressures, but at a rather modest level of fluid compression (2500 psi). This suggests that a maximum possibly exists for the reaction, similar to that observed by Temelli et al. [46] for the glycerolysis of various vegetable oils in a stirred eactor pressurized with SC-CO_2. The fact that the reaction can be made to yield one or more of the desired end products at lower pressure is important, since this minimizes the cost of scaling up the reaction to a production plant level. It is also interesting to note that the reaction may not be dependent on the use of carbon dioxide and that another pressurized fluid might produce the same end result.

The presence of moisture in natural product substrates can have an effect on enzymatic-based synthesis. Previous studies [55] have shown that there is a minimal amount of water that must be associated with the enzyme in the presence of the critical fluid to assure retention of activity. However, excessive water can denature the enzyme, leading to loss

of activity and conversion of reactants. This is illustrated by the results in Table 6 where the effect of added water on the methanolysis of corn oil to form fatty acid methyl esters (FAMES) is described. Note that in terms of volume percent of water in SC-CO_2, that this is quite a small quantity (0.05 vol. %) and must be rigorously controlled to prevent loss of activity and conversion. Fortuitously, as shown in Figure 3, the solubility of water in SC-CO_2 is quite small, and this aids in maintaining the activity of the enzyme for long periods of time when extracting and converting natural product extracts such as triglycerides. Another convenient way of maintaining the hydration level critical for maintenance of the enzyme's activity in the presence of a critical fluid is to add the requisite amount of water via a syringe pump into the critical fluid.

TABLE 6. Effect of added water on the methanolysis of corn oil in SC-CO_2.

Volume % Water in Carbon Dioxide	Relative Activity
0	100
0.05	99
0.10	81
0.20	56
0.30	18

The addition of reactants to a flow reaction system operating under critical fluid conditions can be quite critical in assuring maximum yield of end-products. For example, Jackson and King [50] have shown that the addition of methanol for conducting a transesterification of a vegetable oil must be optimized, or the relative activity of the enzyme will not be realized. This is required to assure that there is a adequate stoichiometry of the reactants as well as time for these moieties to react during their passage over the supported enzyme catalyst.

Another type of reaction that can be catalyzed by an enzyme in the presence of a supercritical fluid, that has commercial potential, is the interesterification of vegetable oils to produce a "randomized" product having quite different physical and chemical properties than the starting materials. Jackson et al. [52] have interesterified a variety of starting materials by dissolving them in SC-CO_2 and transporting them over immobilixed beds of

Novozym SP-435 lipase at 27.5 MPa and 65°C. The end effect is quite striking since liquid vegetable oil feedstocks can be randomized to products of a semi-solid nature having potential as margin base stocks. Figure 21 shows this effect where the solid fat index (a measure of the ratio of liquid to solid fat content) is plotted versus temperature for a randomized palm olein that has been converted by passage at two different pressures (4000, 5000 psi) at 65°C over Novozym SP 435. When these products are characterized with the starting (natural) palm olein with respect to the temperature dependency of the solid fat index, it can be seen that at temperatures in excess of 20°C, the randomized oils have more "solid-like" properties than the starting palm olein.

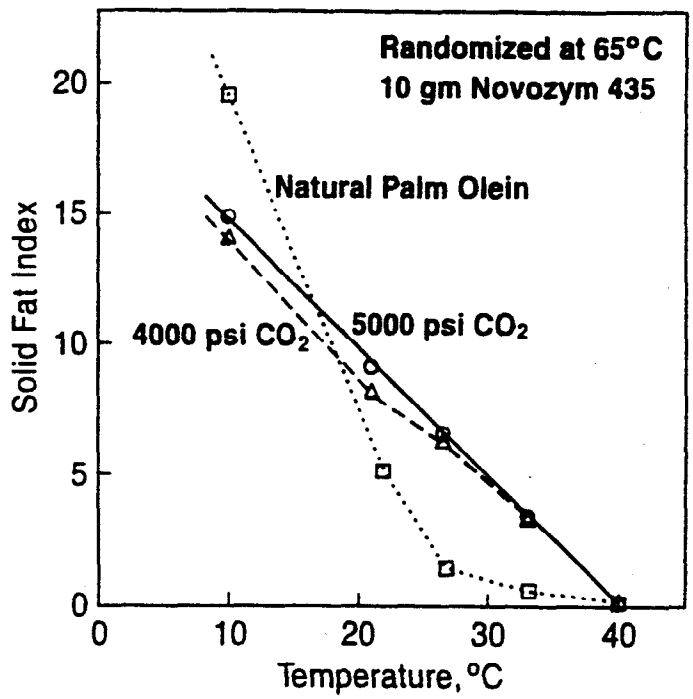

Figure 21. Solid fat index (SFI) vs temperature for SFR-randomized palm olein and native palm olein.

Figure 22 illustrates some other parameters that must be considered when randomizing oil and fat mixtures via reaction in SC-CO_2. Here the dropping point (another measure of the solids content of a oil/fat mixture) is plotted as a function of catalyst charge for the rearrangement of palm olein in a tubular bed reactor. Likewise, the relationship between catalyst charge and product throughput is also illustrated. Figure 22 shows that there is a

dependence between the dropping point and catalyst charge, and that more catalyst is needed to affect a higher dropping point value. However, the inverse relationship is observed for the relationship between the catalyst charge and the product throughput. This illustrates that in some cases there are tradeoffs that must be considered in conducting synthesis under supercritical fluid conditions, and for example, one must sacrifice throughput at the expense of achieving a higher dropping point characteristic in the final-derived product.

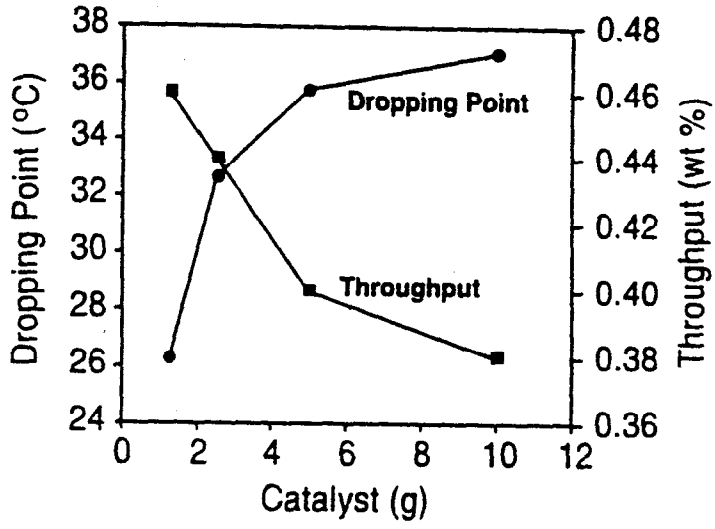

Figure 22. Effect of catalyst concentration on fat dropping point and throughput of randomized palm olein via SFR and lipase catalysis.

In the studies cited to date, SC-CO_2 has been the predominant critical fluid media that has been used, in deference to its benign environmental impact and compatibility with the processing of food-related agricultural products. Another medium that meets these criteria is subcritical water; that is hot compressed water held between 1 -218 atmospheres and its normal boiling point and critical temperature of 374°C. Several studies [55,56] have shown that water under these conditions can be utilized as a reaction medium, both for non-degradative/degradative reactions as well as hydrolysis. Research conducted in our laboratory has utilized subcritical water for the hydrolysis of vegetable oils to synthesize fatty acid mixtures [56]. The results in Table 7 demonstrate how complete this conversion

can be under a variety of conditions. Note that residence times under ten minutes can give over 99% conversion of the vegetable oil feedstock (in this case soybean oil) to the component fatty acids. This approach uses higher water to oil feed ratios into a flow reactor then currently used in industrial hydrolysis processes [57], however the conversions listed in Table 7 were accomplished in a open tubular reactor and required no catalyst.

TABLE 7. Conversion of soybean oil to free fatty acids using subcritical water in a open tubular flow reactor.

Residence Time (min)	12.6	9.9	7.5
Temperature (°C)	335	335	335
Pressure (atm)	125	125	134
Water:Oil Ratio	2.5:1	5:1	2.5:1
% Free Fatty Acid Yield	98	100	90.4

5. Final Overview in Applying Supercritical Fluid Technology to the Processing of Agrimaterials and Natural Products

In the proceeding sections we have demonstrated how critical fluids, applied as an overall technological approach, can be extremely useful in isolating, fractionating, and converting agriculturally-derived products into useful industrial products. The high capital costs of implementing critical fluid technology makes it imperative that plants and processing facilities be adaptable to other roles besides just the extraction mode. The examples presented suggest such options and show that SF. and SFR can also be accomplished using benign critical fluid media and conditions compatible with the end use of products for human consumption or further industrial use.

For example, we have alluded to the possibility of synthesizing monoglyceride-enriched mixtures utilizing supercritical fluid media [46,51]. This can be accomplished in the presence of SC-CO_2 using a stirred reactor at the high temperatures conventionally used for glycerolysis, but in the absence of a catalyst. Another synthetic option, is to use enzyme-catalyzed glycerolysis in a flow reactor to produce glyceride mixtures containing

in excess of 90 wt. % monoglycerides [51]. For both of the above synthetic options, it is possible to achieve further enrichment of the monoglyceride product mixture by subjecting it to SFF via the thermal gradient fractionation approach described in Section 3.2.2. This concept is illustrated in Figure 23 where one of the above two SFR options could proceed to a separation stage (SF.) integrated into the overall production scheme. Note that the bottom product of the thermal gradient fractionating tower separation (excess diglycerides {DAGs} and triglycerides {TAGs}) can be recycled back to the reaction stage (SFR) for further conversion to monoglycerides (MAGs). This is but one of several critical fluid based processes that can be combined in an overall production scheme.

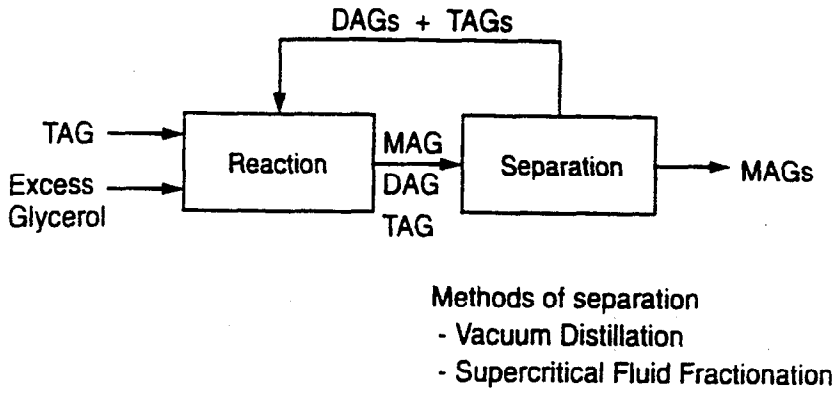

Figure 23. Glycerolysis reaction coupled with SFF of resultant glyceride mixture.

Figure 24 shows other possibilities for linking up these individual critical fluid-based options into tandem processes. Here the previously discussed option is shown initially as well as the supercritical fluid extraction and chromatographic separation of phospholipids which was noted in Section 3.2.3. Also, our previously-cited example of subcritical water synthesis of fatty acids from natural oil feedstocks is noted, the end product in this case is a mixture of fatty acids contained in an aqueous emulsion. These can be separated from water via a membrane process or counter currently into supercritical or liquid carbon dioxide. Further rectification of the fatty acid mixtures would also be amenable to fractionation via the thermal gradient fractionation column mentioned previously.

Recently we have combined two reaction sequences in supercritical media to produce aliphatic fatty alcohol mixtures for the surfactant oleochemical market. This is accomplished by using a transesterification step to synthesize fatty acid methyl esters

(FAMES) from the vegetable oil [50], and then transporting this product in SC-CO_2 into a hydrogenation reactor. Hydrogenation of the FAMES is then accomplished by flowing either a binary mixture of hydrogen with either carbon dioxide or propane, thereby facilitating the total reduction of the FAMES to the saturated alcohols and methanol. The methanol by-product can then be fed back into the first stage of the synthesis process to produce the FAMES. Other combinations of SFE, SF. and SFR do exist and offer some intriguing options for production of materials from complex natural substrates.

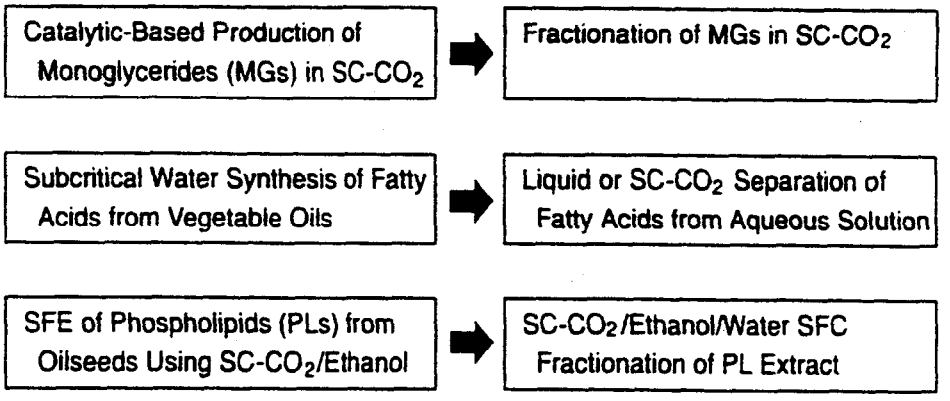

Figure 24. Processing options and combinations utilizing critical fluids.

Finally, "field side" processing in which the critical fluid processing unit would be house in close proximity to the agricultural resource (e.g., field) is within the capabilities of current state of critical fluid technology. This is an important option to the processor of natural materials, particularly when these materials are subject to degradation of valuable components, during transport to a distant processing facility. Such a "green" processing facility offers several other advantages, including an environmentally-compatible production method, savings in transportation costs, and the ability to recycle by-product streams in agricultural setting. Portable critical fluid production units do exist [58] and the author knows of one existing facility that has been constructed in a barn on a agricultural estate in Southern England.

References

1. McHugh, M.A. and Krukonis, V.J. (1994) *Supercritical Fluid Extraction -2nd Ed.*,Butterworth-Heinemann. Boston, MA, USA, pp. 294-299.

2. King, M.B. and Bott, T.R. (1993) *Extraction of Natural Products Using Near-Critical Solvent*, Blackie Academic & Professional, Glasgow, Scotland, pp. 84-100.

3. King, J.W., Holliday, R.L., Sahle-Deemessie, E., Eller, F.J., and Taylor, S.L. (1997) Extraction, fractionation, and reaction of agricultural, food and natural products utilizing supercritical carbon dioxide and cosolvent mixtures. *Proceedings of the 4th International Symposium on Supercritical Fluids - Vol. C*, Tohoku University Press, Sendai, Japan, pp. 833-838.

4. Schneider, G. (1994) Phase equilibria in fluid systems. *Ber. Bunsenges Phys. Chem.* **88**, 841-848.

5. Ref. 1, p. 368.

6. Stahl, E., Quirin, K.-W., and Gerard, D. (1988) *Dense Gases for Extraction and Refining*, Springer-Verlag, Heidelberg, Germany, p. 131.

7. Cygnarowicz-Provost, M., King, J.W., Marmer, W.N., and Magidman, P. (1994) Extraction of wool grease with supercritical carbon dioxide. *J. Am. Oil Chem. Soc.*, 71, 223-225.

8. Bowman, L.M. (1976) *Dense Gas-Chromatographic Studies*. Ph.D. Thesis, University of Utah, Salt Lake City, UT.

9. Ref. 1, pp. 24-25.

10. Giddings, J.C., Myers, M.N., and King, J.W. (1969) Dense gas chromatography at pressures to 2000 atmospheres. *J. Chromatogr. Sci.* 7, 276-283.

11. King, J.W. and Friedrich, J.P. (1990) Quantitative correlations between solute molecular structure and solubility in supercritical fluids. *J. Chromatogr.* **517**, 449-458.

12. Stahl, E., Schilz, W., Schultz, E., and Willing, E. (1980) A quick method for the microanalytical evaluation of the dissolving power of supercritical gases, in G.M. Schneider, E. Stahl, and G. Wilke (eds.), *Extraction with Supercritical Gases*, Verlag Chemie, Weinheim, Germany, pp. 93-114.

13. Ref. 1, p. 301.

14. Evelein, K.A., Moore, R.G., and Heidemann, R.A. (1976) Correlation of the phase behavior in the systems hydrogen sulfide-water and carbon dioxide-water. *Ind. Eng. Chem., Process Des. Dev.* **15**, 423-428.

15. Favati, F., King, J.W., and Mazzanti, M. (1991) Supercritical carbon dioxide extraction of evening primrose oil. *J. Am. Oil Chem. Soc.* **68**, 422-427.

16. Hong, I.K., Rho, K.S., Lee, K.S., Lee, W.H., and Yoo, K.P. (1990) Modeling of soybean oil extraction with supercritical carbon dioxide. *Korean J. Chem. Eng.*, 7, 40-46.

17. Perrut, M., Clavier, J.Y., Poletto, M., and Reverchon, E. (1997) Mathematical modeling of sunflower seed extraction by supercritical CO_2. *Ind. Eng. Chem. Res.* **36**, 430-435.

18. Poletto, M. and Reverchon, E. (1996) Comparison of models for supercritical fluid extraction of seed and

essential oils in relation to the mass-transfer rate. *Ind. Eng. Chem. Res.*, **35**, 3680-3686.

19. King, J.W., Cygnarowicz-Provost, M., and Favati, F. (1997) Supercritical fluid extraction of evening primrose oil: Kinetic and mass transfer effects. *Ital. J. Food Sci.* **9**, 193-204.

20. Favati, F., Florentini, R.and De Vitis, V. (1994) Supercritical fluid extraction of sunflower oil: Extraction dynamics and process optimization, *Proceedings of the 3rd International Symposium on Supercritical Fluids*, Institut National Polytechnique de Lorraine, Nancy, France, pp. 305-310.

21. King, J.W., Johnson, J.H., and Friedrich, J.P. (1989) Extraction of fat tissue from meat products with supercritical carbon dioxide. *J. Agric. Food Chem.* **37**, 951-954.

22. Goodrum, J.W. and Kilgo, M.B. (1989) Rapeseed oil recovery by CO_2 solvent: Recovery, kinetics and extraction model. *Trans. Am. Soc. Agric. Engs.* **32**, 727-731.

23. Eggers, R. (1996) Supercritical fluid extraction (SFE) of oilseeds/lipids in natural products, in J.W. King and G.R. List (eds.), *Supercritical Fluid Technology in Oil and Lipid Chemistry*, AOCS Press, Champaign, IL, USA, pp. 35-64.

24. King, J.W.(1997) Critical fluids for oil extraction, in P.J. Wan and P.J. Wakelyn (eds.), *Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils*, AOCS Press, Champaign, IL, USA, pp. 283-310.

25. Foveate, F., King, J.W., Friedrich, J.P., and Eskins, K. (1988) Supercritical CO_2 extraction of carotene and lutein from leaf protein concentrates. *J. Food Sci.* **53**, 1532-1536.

26. Montanari, L., Sensidoni, A. and Kikic, I. (1997) Supercritical fluid extraction of mixtures containing triglycerides and sterols, in E. Reverchon (ed.). *Proceedings of the 4th Italian Conference on Supercritical Fluids and Their Applications*, Capri, Italy, pp. 89-95.

27. Montanari, L., King, J.W., List, G.R., and Rennick, K.A. (1996) Selective extraction of phospholipid mixtures by supercritical carbon dioxide and cosolvents. *J. Food Sci.* **61**, 1230-1233, 1253.

28. Brunner, G. (1994) *Gas Extraction*, Steinkoff, Darmstadt, Germany.

29. Brunner G. (1998) Processing of natural materials by supercritical gases, *Proceedings of the 5th Meeting on Supercritical Fluids*, Institut National Polytechnique, Nancy, France, pp. 413-424.

30. Hagashidate, S., Yamauchi, Y., and Saito, M. (1994) Fractionation of eicosapentaenoic acid and docosahexaenoic acid esters from esterified fish oil by coupled supercritical fluid extraction-chromatography, in M. Saito, Y. Yamauchi, and T. Okuyama (eds.). *Fractionation by Packed Column SFC and SFE*, VCH Publishers, New York, NY, USA, pp. 191-203.

31. Sato, M., GOTO, M., Kunishima, N., Kodama, A. and Hirose, T. (1997) Pressure swing adsorption process in supercritical carbon dioxide for the fractionation of citrus oil, *Proceedings of the 4th International Symposium on Supercritical Fluids - Vol. B*, Tohoku University Press, Sendai, Japan, pp. 629-632.

32. Reverchon, E. (1997) Supercritical fluid extraction and fractionation of essential oils and related products. *J. Supercrit. Fluids*, **10**, 1-37.

33. Rizvi, S.S.H., Chao, R.R. and Liaw, Y.J. (1988) Concentration of omega-3 fatty acids from fish oil using supercritical carbon dioxide, in B.A. Charpentier and M.R. Sevenants (eds.), *Supercritical Fluid Extraction and Chromatography*, American Chemical Society, Washington, DC, USA, pp. *89-108.*

34. Dunford, N.T. and Temelli, F. (1995) Extraction of phospholipids from canola with supercritical carbon

dioxide and ethanol. *J. Am. Oil Chem. Soc.*, 72, 1009-1015.

35. List, G.R., King, J.W., Johnson, J.H., Warner, K., and Mounts, T.L. (1993). Supercritical CO_2 degumming and physical refining of soybean oil. *J. Am. Oil Chem. Soc.* **70**, 473-476.

36. Eisenbach, W. (1984) Supercritical fluid extraction. A film demonstration. *Ber. Bunsenges. Phys. Chem.* **88**, 882-887.

37. Nelson, W.B., Gauglitz, E.J., Hudson, J.K., Stout, V.F., and Spinelli, J. (1988) Fractionation of menhaden oil ethyl esters using supercritical fluid CO_2. *J. Am. Oil Chem. Soc.* **65**, 109-117.

38. King, J.W., Sahle-Demessie, E., Temelli, F. and Teel, J.A. (1997) Thermal gradient fractionation of glyceride mixtures under supercritical fluid conditions. *J. Supercrit .Fluids* **10**, 127-137.

39. Jackson, M.L. (1998) Monoglyceride production via enzymatic glycerolysis of oils in supercritical CO_2. *U.S. Patent 5, 747, 305.*

40. Sahle-Demessie, E. (1997) Fractionation of glycerides using supercritical carbon dioxide. *Ind. Eng. Chem. Res.* **36**, 4906-4913.

41. Giddings, J.C., (1991) *Unified Separation Science*, John Wiley & Sons, Inc., New York, NY, USA.

42. King, J.W., Foveate, F., and Taylor, S.L. (1996) Production of tocopherol concentrates by supercritical fluid extraction and chromatography. *Sep. Sci. Tech.* **31**, 1843-1857.

43. Montanari, L., King, J.W., List, G.R., and Rennick, K.A. (1994) Selective extraction and fractionation of natural phospholipid mixtures by supercritical CO_2 and cosolvent mixtures, in G. Brunner and M. Perrut (eds.), *Proceedings of the 3rd International Symposium on Supercritical Fluids - Vol. 2*, Institut National Polytechnique de Lorraine, Nancy, France, pp 497-504.

44. Montanari, L., Fantozzi, P., Snyder, J.M., and King, J.W. (1997) Selective extraction of phospholipids from soybeans with supercritical carbon dioxide, in E. Reverchon (ed.), *Proceedings of the 4th Italian Conference on Supercritical Fluids and Their Applications*, Capri, Italy, pp.81-88.

45. Clifford, A.A. (1994) Reactions in supercritical fluids, in E. Kiran and J.M.H. Levelt Sengers (eds.), *Supercritical Fluids, Fundamentals for Application*, Kluwer Academic Publishers, Dordrecht, Germany, pp. 449-479.

46. Temelli, F., King, J.W., and List, G.R. (1996) Conversion of oils to monoglycerides by glycerolysis in supercritical carbon dioxide media. *J. Am. Oil Chem. Soc.* **73**, 699-706.

47. Aaltonen, O. and Rantakyla, M. (1991) Biocatalysis in supercritical CO_2. *Chemtech*, **21**, 240-248.

48. Gunnlaugsdottir, H. and Sivik, B. (1995) Lipase-catalyzed alcoholysis of cod liver oil in supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* 72, 399-405

49. King, J.W., Jackson, M.A., List, G.R., Sahle-Demessie, E., Holliday, R.L., and Temelli, F. (1998) Synthetic modification of seed-derived oils utilizing reaction chemistry in supercritical fluids., in S.S. Koseoglu, K.C. Rhee, and FR.F. Wilson (eds.), *Proceedings of the World Conference on Oilseed and Edible Oils Processing*, AOCS Press, Champaign, IL., USA, pp. 314-320.

50. Jackson, M.A. and King, J.W. (1996) Methanolysis of seed oils in flowing supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* 73, 353-356.

51. Jackson, M.A. and King, J.W. (1997) Lipase-catalyzed glycerolysis of soybean oil in supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* 74, 103-106.

52. Jackson, M.A., King, J.W., List, G.R., and Neff, W.E. (1997) Lipase-catalyzed randomization of fats and oils in flowing supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* 74, 635-639.

53. Snyder, J.M., King, J.W., and Jackson, M.A. (1996) Fat content for nutritional labeling by supercritical fluid extraction and an on-line lipase catalyzed reaction. *J. Chromatogr.*, 750, 2101-2107.

54. Randolph, T.W., Blanch, H.W., and Clark, D.S. (1991) Biocatalysis in supercritical fluids, in J.S. Dordick (ed.). *Biocatalysis for Industry*, Plenum Press, New York, NY, USA, pp. 219-237.

55. Kabyemela, B.M., Takigawa, M., Adschiri, T., Malaluan, R.M., and Arai, K. (1997) Mechanism and kinetics of cellobiose decomposition in sub and supercritical water, in *Proceedings of the 4th International Symposium on Supercritical Fluids - Vol. B*, Tohoku University Press, Sendai, Japan, pp. 547-550.

56. Holliday, R.L., King, J.W., and List, G.R. (1997) Hydrolysis of vegetable oils in sub- and supercritical water. *Ind. Eng. Chem. Res.* 36, 932-935.

57. Barneby, H.L. and Brown, A.C. (1948) Continuous fat splitting plants using the Colgate-Emery process. *J. Am. Oil Chem. Soc.*, 25, 95-99.

58. CF Systems Organics Extraction Process: (1990) New Bedford Harbor, MA. *EPA Report No. /540/A-5-90/002*, U.S. Environmental Protection Agency, Cincinnati, OH, USA.